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**Localized (5  $\mu$ m) Probing and Detailed Mapping of Hair with  
Synchrotron Powered FT-IR Microspectroscopy**

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# Localized (5 $\mu\text{m}$ ) Probing and Detailed Mapping of Hair with Synchrotron Powered FT-IR Microspectroscopy

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The thickness and high absorptivity of single hairs typically result in the saturation of major infrared bands and their distortion. Single human hairs longitudinally microtomed and mounted on mirror slides were scanned routinely in the past with a 20  $\mu\text{m}$  x 100  $\mu\text{m}$  aperture that limited spatial resolution for localized probing and detailed mapping. Use of the nondivergent, bright, and low-noise synchrotron source for FT-IR microspectroscopy enables good S/N even at apertures as small as 5-6  $\mu\text{m}$ . Functional group mapping as well as localized probing for extraneous materials illustrates the utility of this powerful probe.

## INTRODUCTION

Human hair is a naturally occurring fiber that is of particular interest in pathology and in forensic science. FT-IR microspectroscopy of hair has provided information valuable in matching hair found at one location with the source of that hair. Material placed on the surface of the hair has been found by use of surface techniques such as attenuated total reflectance (ATR). In such cases, the hair composed largely of protein has provided a suitable substrate for absorbing or adsorbing other materials. Interesting presentations have been made of data produced from human hair subjected to a variety of cosmetics (1). Hair is also a very good adsorber for materials in the atmosphere such as solvents, smoke, and various odors. The probing discussed here is of the internal composition of the hair rather than materials that are found on the surface. When probing for the existence of foreign substances that have been placed in the human body, their uptake in hair is of concern. With this process, nature also provides a useful analytical concentration step.

## PREVIOUS MICROSPECTROSCOPY

Traditionally, spectroscopists serving the area of pathology have separated the internal material from that found on the surface usually by a procedure of first washing the surface to remove contamination and then microtoming the sample in a block of paraffin to permit the usage of transmission reflection microspectroscopic techniques. Prior to using synchrotron powered FT-IR microspectroscopy, heroic efforts were made to obtain spectra of hair sliced in embedded paraffin that was placed on a reflecting microscope slide (2,3). Localization of chemicals making up the hair and potential localization of any foreign materials was limited by the signal-to-noise and thus a relatively large aperture was required. Typically, an

aperture of 20  $\mu\text{m}$  x 100  $\mu\text{m}$  was used to obtain signal quantity and spectral quality that were dependable. Profiling of one particular hair was done by stepping the aperture in small increments across the width of the hair. Small (5  $\mu\text{m}$ ) steps of the 20  $\mu\text{m}$  wide aperture did produce interesting information regarding the distribution of the materials that make up the hair. In particular, the protein bands were stronger in the center of the hair, near the medula, than they were out at the cortex (3). In cases where a foreign substance was present, it was necessary to use spectral subtraction of the spectra of control samples in order to locate or detect the presence of a foreign chemical substance.

## SYNCHROTRON SOURCE

Localized probing and detailed mapping of hair was considered to be a good candidate for synchrotron powered FT-IR microspectroscopy. The critical requirement for these measurements is high signal to noise from a small illuminated spot on the sample, small being a few microns. The high signal to noise of the synchrotron source is apparent from Fig. 1. Infrared synchrotron radiation is a broadband source that is about 1000 times brighter than standard thermal sources (4). It is also in principle more stable, because no thermal fluctuations occur in this type of source. Light is generated by the electric field experienced as bunches of electrons circulate at relativistic energies in a storage ring. The intensity is proportional to the number of electrons, which is constant apart from a slowly varying exponential loss, so if the electron orbit is stable, the intensity is also constant. Brightness is crucial for microscopy where one is trying to illuminate as small an area as possible with as much light as possible. In the case of the synchrotron, the source size is roughly 1 mm by 1 mm, and the radiation is emitted into an angle of approximately 10 milliradians by 10 milliradians so

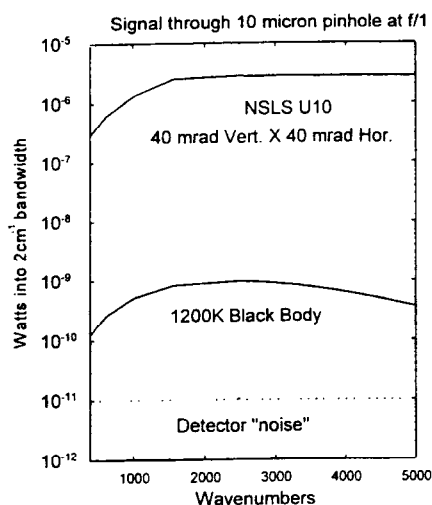


Figure 1. Signal of synchrotron vs. black body (5).

that the emittance is about  $10^{-4}$  mm<sup>2</sup> steradians. In contrast, the emittance for a thermal source is more than  $10^{-2}$  mm<sup>2</sup> steradians, so only 0.001% of the light from the thermal source would be available to illuminate a 10  $\mu$ m sized spot on the sample. In practice with a thermal source, one requires illuminated areas of about 30  $\mu$ m in order to obtain reasonable signal to noise ratio, and even then one has to average over many scans. A synchrotron illuminated infrared microscope facility was constructed at beamline U2B of the National Synchrotron Light Source at Brookhaven National Laboratory (6). Light from the synchrotron was collimated and introduced into a standard commercial FT-IR microspectrometer (IR $\mu$ s, Spectra-Tech, Shelton, CT) by simply removing the collimating mirror for the thermal source. The overall optical system had some aberrations, so that the gain over the thermal source was a factor of about 50. This facility has served well both for material characterization and for use on biological substances or tissue. Localization is important not just to deal with small samples in the field of the microscope but to isolate subsamples within the microscopic field and to interrogate those without accidental sampling of neighboring tissue. The Spectra-Tech IR $\mu$ s microspectrometer is doubly confocal, meaning that it has an aperture before the objective and another after the condenser, in the case of transmission, to limit the part of the field being interrogated and to reject the sampling of neighboring tissue brought about by diffraction. In practice, with the synchrotron source, we were able to obtain good signal to noise ratio when sampling areas whose size was of the order of the wavelength of the light being used, i.e. a few microns, in sampling times of less than a minute per sampled area.

## EXPERIMENTATION AND RESULTS

With the synchrotron source and an IR $\mu$ s microspectrometer (Spectra-Tech, Shelton, CT), probing of

transmission reflection paraffin-microtomed samples of single human hairs on a mirror surface was attempted. Of the first six specimens with which this was attempted, only two were sufficiently thin to allow a double transmission. A rectangular mapping experiment with a 12 x 12  $\mu$ m aperture (Fig. 2) showed maximum protein in the center of the hair as previously shown (2). Figure 3 shows a higher density of organic matter in the center and the effect of paraffin contamination on either side of the hair. Attempts to prepare thinner paraffin sections were wholly unsatisfactory. The localized probing reported here was done ultimately by removing thin sections of hair from the microtomed slab of paraffin and mounting them between barium fluoride disks 2 mm thick x 13 mm diameter in a microcompression cell. These specimens were probed using a transmission mode of operation with a 32X objective at a resolution of 4 or 8 cm<sup>-1</sup> with 32-128 scans coadded. Apertures of 12 x 12  $\mu$ m were used to obtain individual spectra. They also were used to step longitudinally along the fiber or across the fiber. The excellent spectrum in Fig. 4 was from a 12 x 12  $\mu$ m aperture with 32 scans coadded. This represents an improvement of the previous requirement of a large 20 x 100  $\mu$ m aperture. Figure 5 shows a series of spectra mapped across a hair also

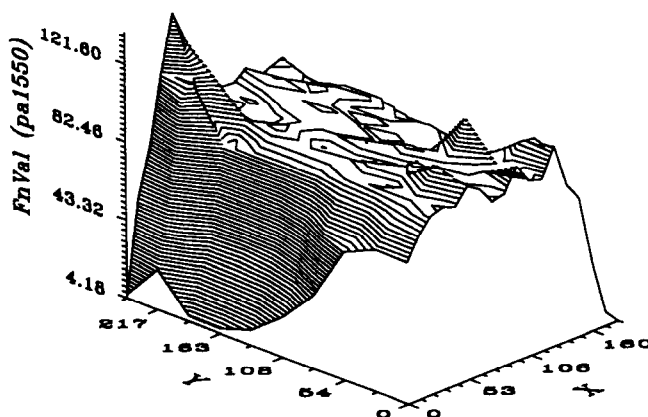


Figure 2. Peak area of 1550 cm<sup>-1</sup> showing distribution of protein

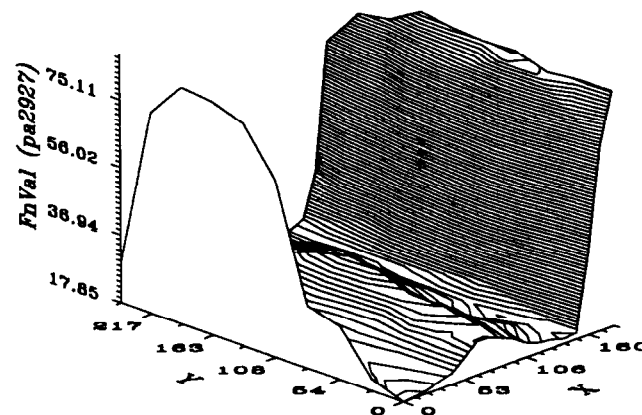


Figure 3. Peak area of 2927 cm<sup>-1</sup> showing density of organic material

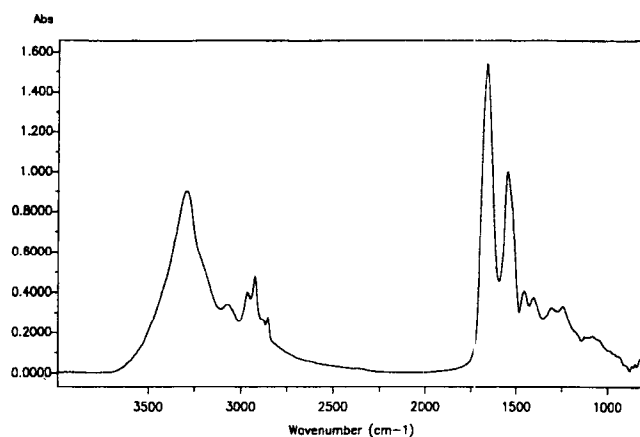


Figure 4. Spectrum obtained with 12 x 12  $\mu\text{m}$  apertures

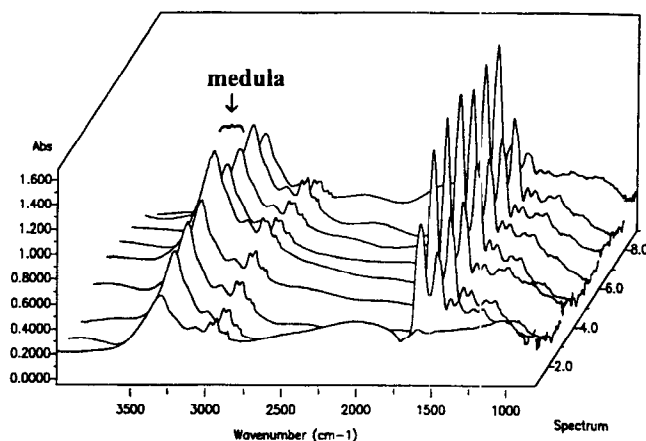


Figure 5. Spectra from a linear map across hair

with a 12 x 12  $\mu\text{m}$  aperture. For specimens that were sufficiently thin, excellent spectra were produced in this manner. The most extreme case of localization that was done on hair specimens with the synchrotron powered system was that of an approximately 5  $\mu\text{m}$  projected aperture that resulted from the use of a pin hole projected through a 32X objective. With a reasonable number of scans coadded, useful spectra shown resulted. With this aperture, different points along the medula were scanned, as shown in Fig. 6. At a point of interest, points across the hair were scanned. Figures 7 and 8 show typical spectra obtained in this manner from different places across the hair shown in the associated photos. Photographs at different parts of one particular hair specimen show the aperture projected at various sample points. Data from select points show the quality of spectra obtained. Note that at one particular point (Fig. 8), there is a hint of a foreign substance as evidenced by the carbonyl band at 1740  $\text{cm}^{-1}$ . It should be pointed out that this particular specimen was a spiked sample. Once the longitudinal location of this foreign substance was located, several probes were taken across the hair in this region which showed a variance of the carbonyl (Fig. 9). The results of line mapping experiments from outside the medula across the medula to the opposite edge

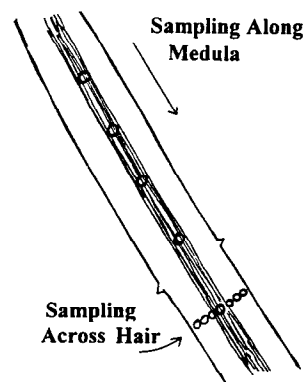


Figure 6. Sketch of individual points scanned

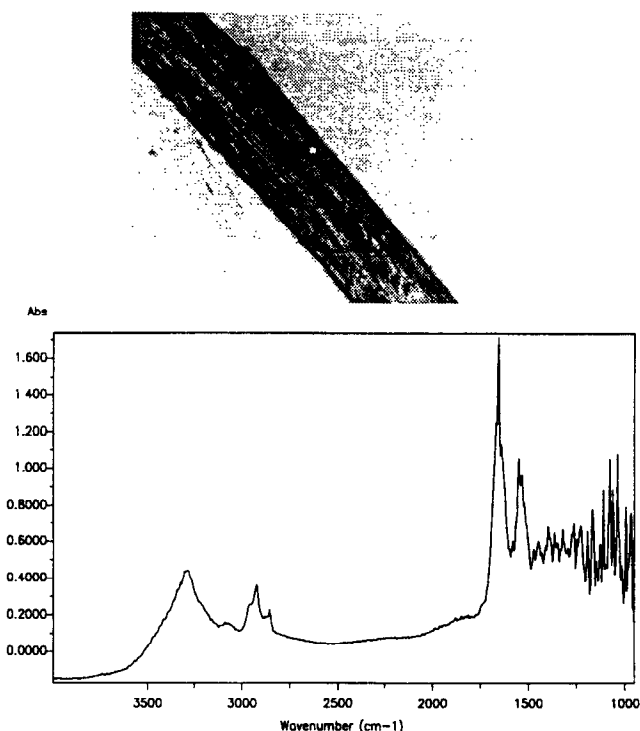
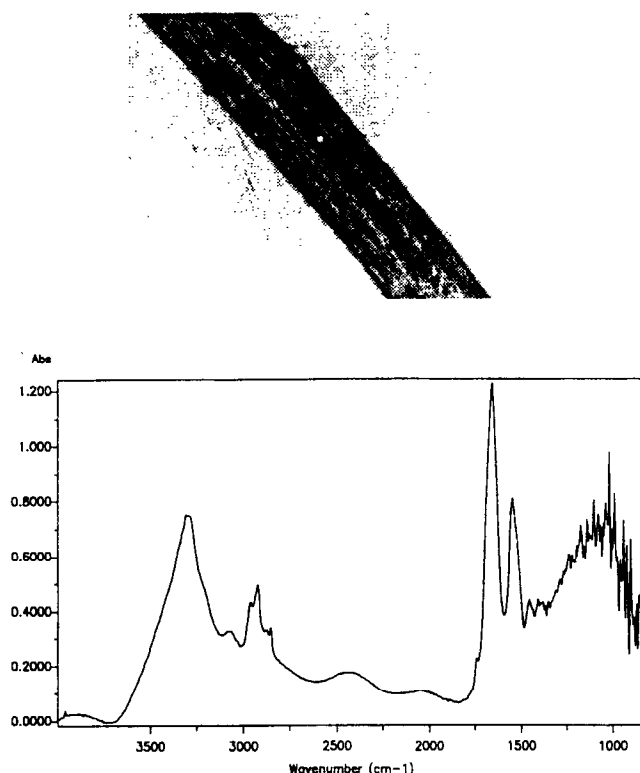


Figure 7. Photograph and corresponding spectrum at a point outside of the medula

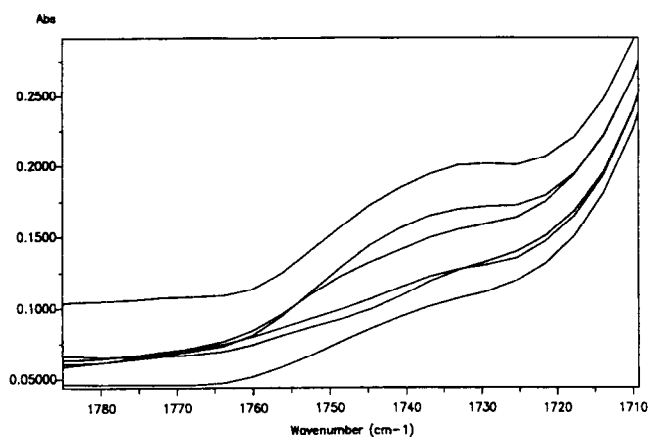
with a 5  $\mu\text{m}$  aperture showed maximum carbonyl peaks across at points 4 and 7 that straddled the two parts in the center of the medula (Fig. 10).

## DISCUSSION AND CONCLUSIONS

The purpose of this work was to demonstrate the degree of localization achievable, the sampling techniques appropriate, and the improvement of localization in such a challenging task as mapping hair. Certainly the ability to change from a 20  $\mu\text{m}$  x 100  $\mu\text{m}$  aperture to a 5  $\mu\text{m}$  circular aperture is a huge improvement in localization. It is also interesting to note that on the longitudinal axis at a typical growth rate for head hair, a 5  $\mu\text{m}$  distance would represent approximately 25 minutes of

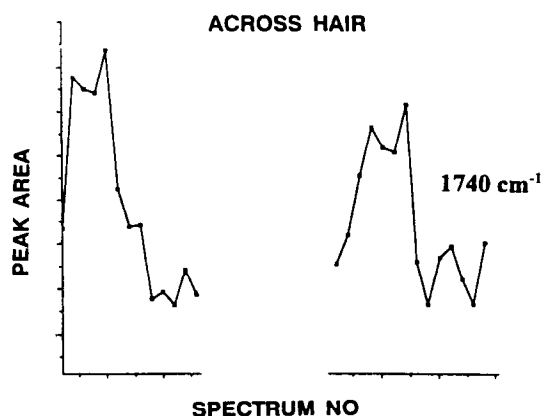


**Figure 8.** Photograph and corresponding spectrum of individual point at the edge of the medulla



**Figure 9.** Spectral region at 1740 cm<sup>-1</sup> showing variation across hair spiked with a drug metabolite

growth. Thus, where the actual hair serves as a recording medium, the microspectroscopy with this spatial resolution then can detect what is being laid down on that substrate in relative units of time. Localized probing of difficult to handle biological specimens is enhanced considerably with synchrotron powered FT-IR microspectroscopy. Excellent spectra resulted from spot sizes as small as 5  $\mu\text{m}$  using a transmission mode for a 6  $\mu\text{m}$  thick cross section between barium fluoride plates in a microcompression cell.



**Figure 10.** Distribution of carbonyl from two line maps across hair spiked with drug metabolite

## ACKNOWLEDGMENTS

Paraffin-embedded microtomed control (drug free) hair specimens were provided by Dr. Kathryn S. Kalasinsky (Armed Forces Institute of Pathology, Washington, DC), whose long-term serious efforts to study the nature of the uptake and deposition of foreign substances in hair during growth inspired the analytical challenge that we accepted. A control hair specimen spiked with a carbonyl-containing major metabolite of a drug was used in the small aperture search for foreign substance localization along the length and across the hair width. This work was supported in part by the National Science Foundation EPSCoR Grant no. OSR-9255223. The National Synchrotron Light Source is supported by the U.S. Dept. of Energy under contract no. DE-AC02-76CH00016.

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